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Technical Report

BACTERIA AT OCEANOGRAPHIC STATIONS OFF SOUTHERN CALIFORNIA – POPULATION DISTRIBUTION IN RELATION TO DEPTH

January 1968

NAVAL FACILITIES ENGINEERING COMMAND

NAVAL CIVIL ENGINEERING LABORATORY

Port Hueneme, California

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bottle, and presterilized Cobet and ZoBell bacteriological samplers (Figures 2 and 3). The bottom water samples from less than 1 foot above the bottom sediment were taken with a specially designed bottom-actuated device which holds and trips a Fjarlie bottle upon contact with the bottom sediment (Figure 4).

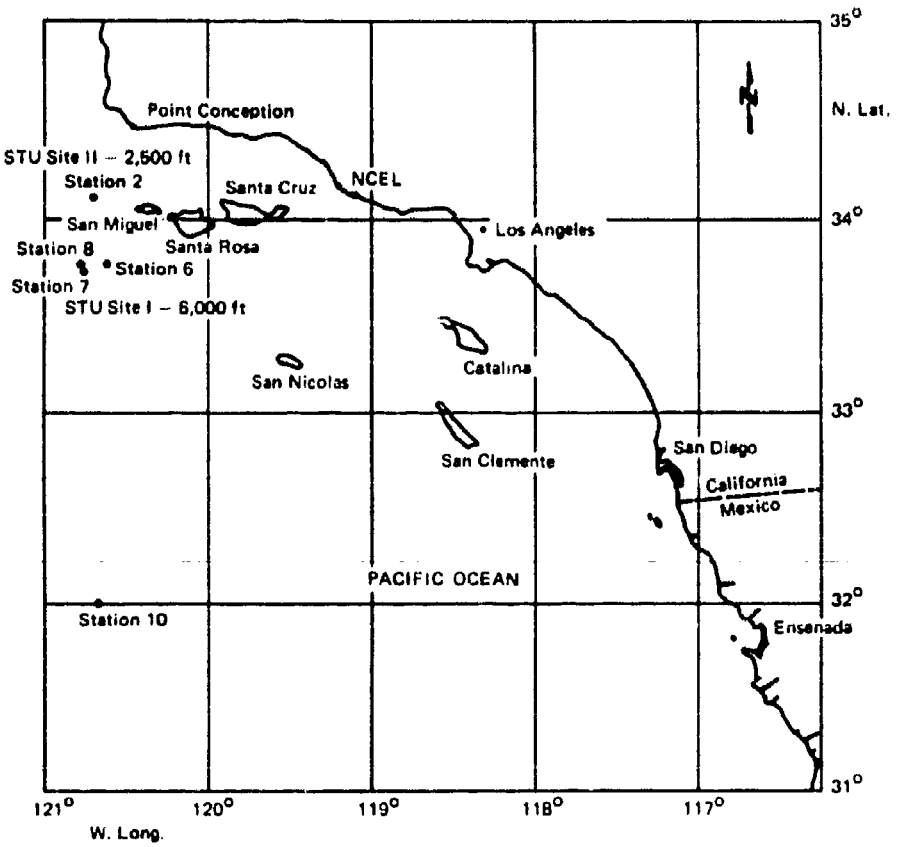

Figure 1. Location of NCEL oceanographic stations where water samples were taken.

These water samplers were attached in series to a hydrographic wire. Several ZoBell bacteriological samplers, which can be triggered in sequence by the messenger released by the next higher sampler on the wire, were placed 3 to 10 feet from the Nansen or Fjarlie bottles. The Cobet bacteriological sampler was placed at the bottom because, although it is triggered by a messenger, it has no facility for releasing a messenger to trip samplers lower on the wire. The bottom-actuated device with a Fjarlie bottle was secured to the hydrographic wire immediately above a 500-pound sinker attached to the end of the wire. The attached water samplers were tripped by sliding a messenger down the wire either at predetermined depths or after contact of the 500-pound sinker with the bottom.

Bacteriological Analysis of Water Samples

As each water sampler was recovered from the sea, it was removed from the hydrographic wire and placed in a holding rack on the deck of the ship. The first sample of water from the sampler was transferred through a clean vinyl tube to a glass bottle used for dissolved oxygen determination. This was to wash away any extraneous bacteria present in the vinyl tube. The remaining seawater sample was transferred either to other glass bottles for salinity and pH measurements or to a sterile milk dilution bottle for bacteriological analysis aboard ship.

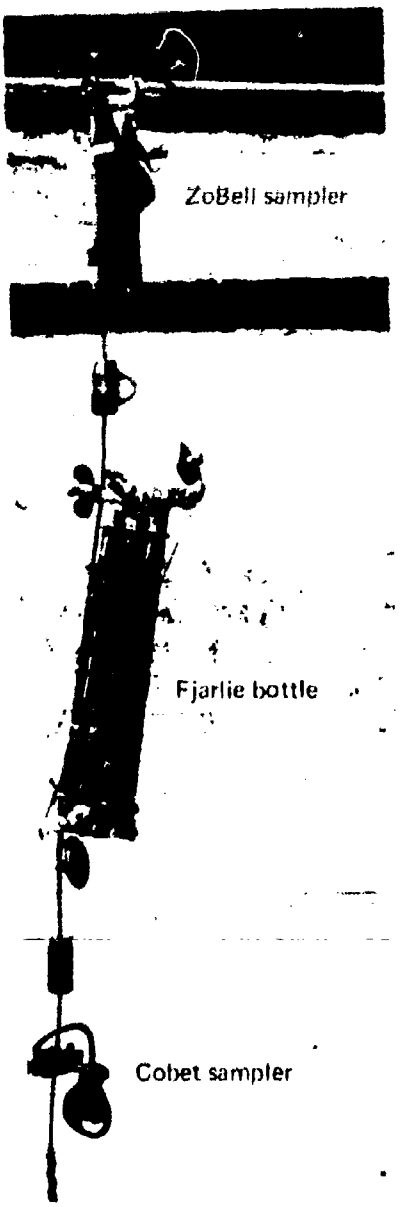

Figure 2.
Fjarlie bottle, and Cobet and ZoBell bacteriological samplers attached to a hydrographic wire.

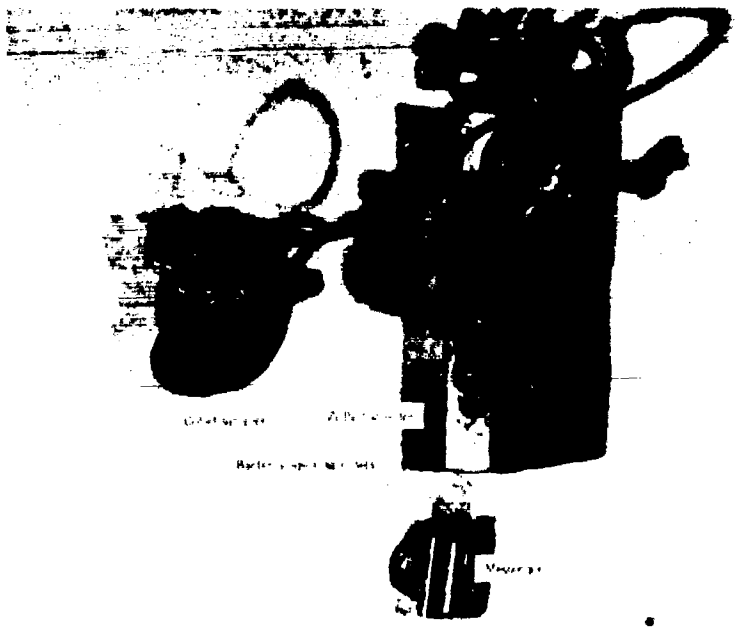

Figure 3. Cobet and ZoBell bacteriological samplers.

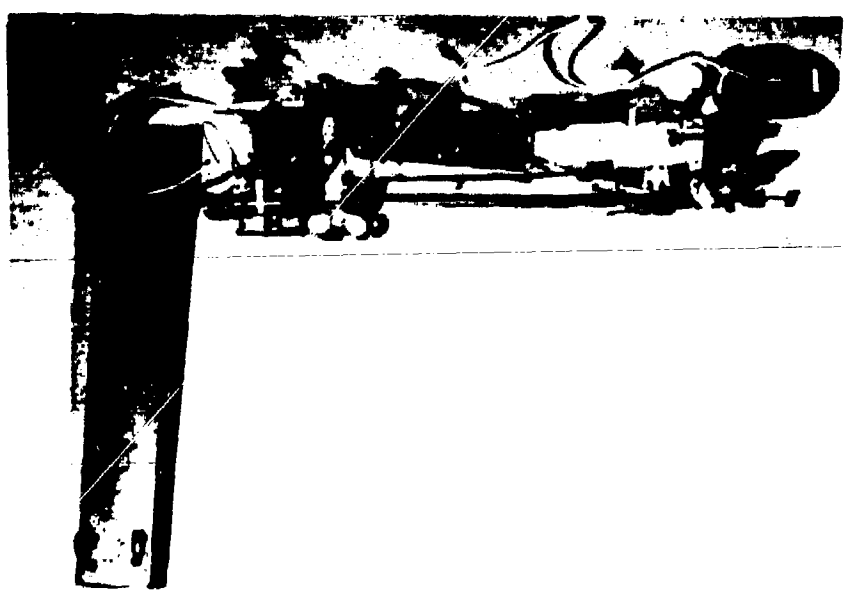

Figure 4. Fjarlie bottle held in a bottom-actuated device designed to collect a seawater sample immediately above the sediment when tripped by weight touching bottom.

The quantity of seawater filtered through a 0.45-micron membrane filter aboard ship depended upon the depth at which the samples were obtained. Samples ranging between 5 to 10 cc were used for surface waters, and 100-cc samples were used for waters at depths of 12,000 feet. On completion of the filtration, the filters were placed on the surface of a nutrient agar medium (2216E) to culture the microorganisms caught on the surface of the filter. This medium was developed by Morita and ZoBell[2] for growing the aerobic marine microorganisms.

The petri dishes containing the membrane filters were stored in the ship's refrigerator. Upon return to the NCEL laboratory, the dishes were transferred to a refrigerator-incubator which was kept at a temperature of 15°C. After 5 to 7 days' incubation, the small bacterial colonies growing on the surface of the filter paper were examined under a stereoscopic microscope, and each colony was counted and recorded. All phases of the bacteriological work were conducted aseptically to prevent extraneous contamination.

The laboratory methods, instruments, and techniques used for determining water temperature, salinity, dissolved oxygen concentration, pH, true depths at which water samples were taken, and other information are described in References 3 and 4.

Bacteriological Analysis of Sediment Samples

The sediment samples taken for bacteriological analysis were obtained with a gravity coring device and a 10-inch-diameter by 3-foot-long steel pipe dredge. The core samples obtained were 1-3/8 inches in diameter and up to 2 feet in length; the pipe dredge produced several pounds of bottom sediment.

As soon as the coring device with the sediment sample was placed on the deck of the ship, the plastic core tube containing the sample was removed and the ends of the tube were sealed with sterile plastic caps. The sediment from the pipe dredge was placed in a plastic bucket and covered with a tight-fitting lid. The samples were then stored in the ship's refrigerator until they were delivered to the NCEL laboratory where the bacteriological analysis was made.

At NCEL, the plastic core tube containing the sample was cut into 3- to 6-inch-long sections with a hack saw. Central portions of the mud from the freshly cut sections were used for bacteriological analysis. Aseptic technique was used during all operations.

A 1-gram sample of each section of sediment was weighed out, and each sample was placed in a test tube containing 9 cc of sterile seawater. The test tubes were then shaken vigorously to obtain uniform suspension.

A 1-cc portion of each suspension was used for inoculating the following media: (1) nutrient medium designated as 2216E developed by Morita and ZoBell[2] was used for growing aerobic bacteria in petri dishes, (2) 0.1 gram of sodium thioglycolate was added to a liter of 2216E medium for growing anaerobic bacteria in Fisher-Spray anaerobic culture dishes, and (3) nutrient medium designated as M10E was used for growing sulfate-reducing bacteria.[2] Approximately 1/2 gram of sediment was placed in a screw-capped test tube filled with medium M10E.

RESULTS

The bacterial population found in the surface waters collected at NCEL test stations ranged from 5 to 100 cells per ml (Figures 5 to 10). This population range is lower than the range recorded near the coastline by ZoBell.[5] He found that the average number of bacteria in surface waters off La Jolla, California (based on monthly observations for 10 years) ranged from 420 to 620 per ml.

Below the immediate surface of the sea, the bacterial population varied randomly with depth and location (Figure 11). The highest bacterial count, 12,000 per 100 ml of seawater, was found at depths between 2,300 to 2,700 feet in the minimum oxygen zone (Figure 8). The lowest number of bacteria, 5 to 10 per 100 ml of seawater, was found at the 12,000-foot depth near the sea floor (Figure 10). The majority of the bacteria collected from the minimum oxygen zone had formed minute, slow-growing colonies on the surface of membrane filters. Under a stereoscopic microscope, these colonies appeared as minute, clear, glistening specks among faster growing larger colonies. These tiny colonies also developed in great numbers on the surface of membrane filters in which seawater samples were taken at depths between 3,600 and 4,600 feet on the same day and a short distance from where the previous water samples were collected (Figure 9).

When the membrane filters with bacterial colonies were stored in a refrigerator at about 5°C for about 2 months, examination revealed that the clear, transparent colonies had become yellow pigmented. To determine the cell morphology of these bacteria, a 48-hour culture was stained with gram stains. When examined under a microscope, the bacterial cells were found to be gram-positive (purple) short rods of uniform shape and size (Figure 12). A physiological test was also conducted to determine if it was a terrestrial form which had become accustomed to living in the marine environment. The bacteria were inoculated into test tubes containing nutrient broth made with distilled water and also into another set of test tubes of nutrient broth made with seawater. This particular species was able to grow in both types of nutrient broth, which indicates that its origin could have been terrestrial.

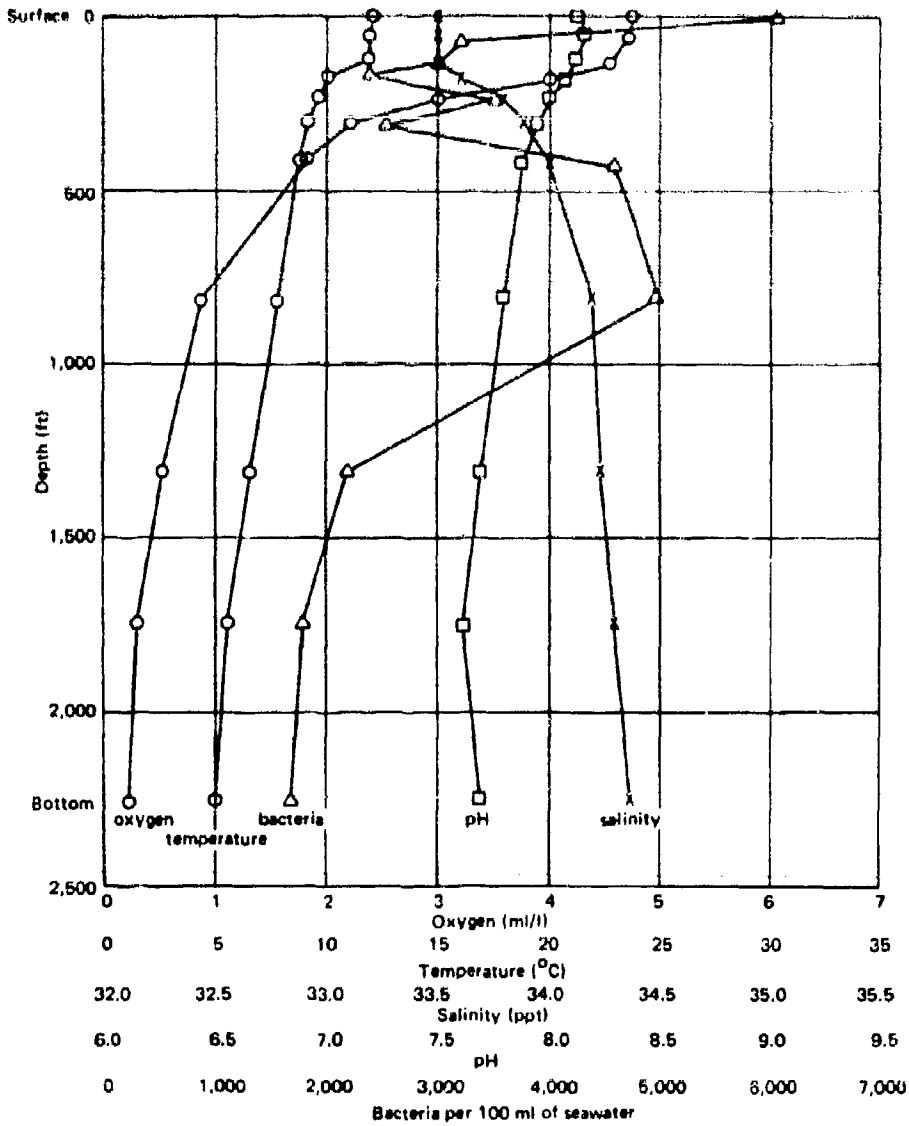

Figure 5. Relationship between vertical distribution of bacteria and environmental parameters (Station 2, Cruise A706-1, June 1967).

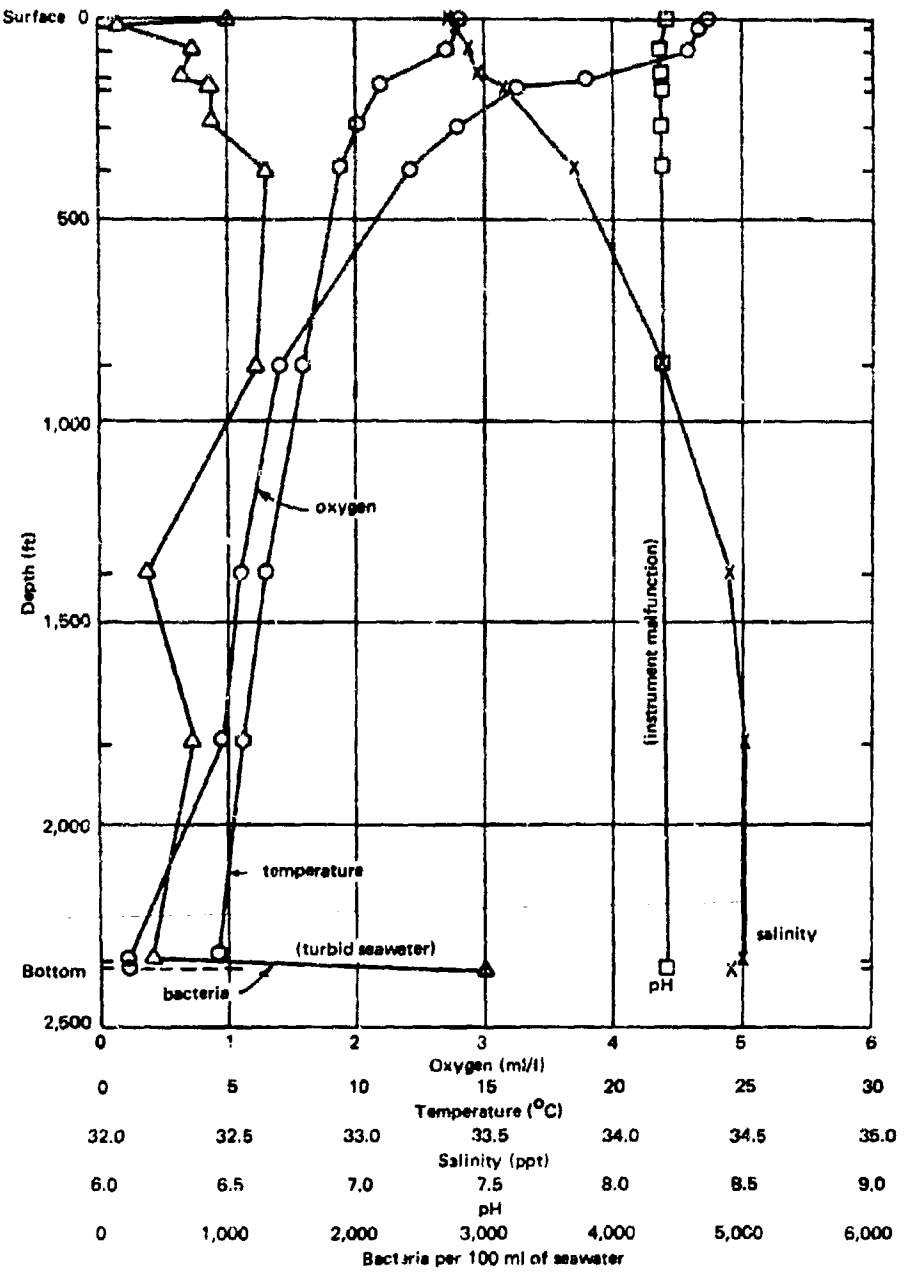

Figure 6. Relationship between vertical distribution of bacteria and environmental parameters (Station 2, Cruise A612-1, December 1966).

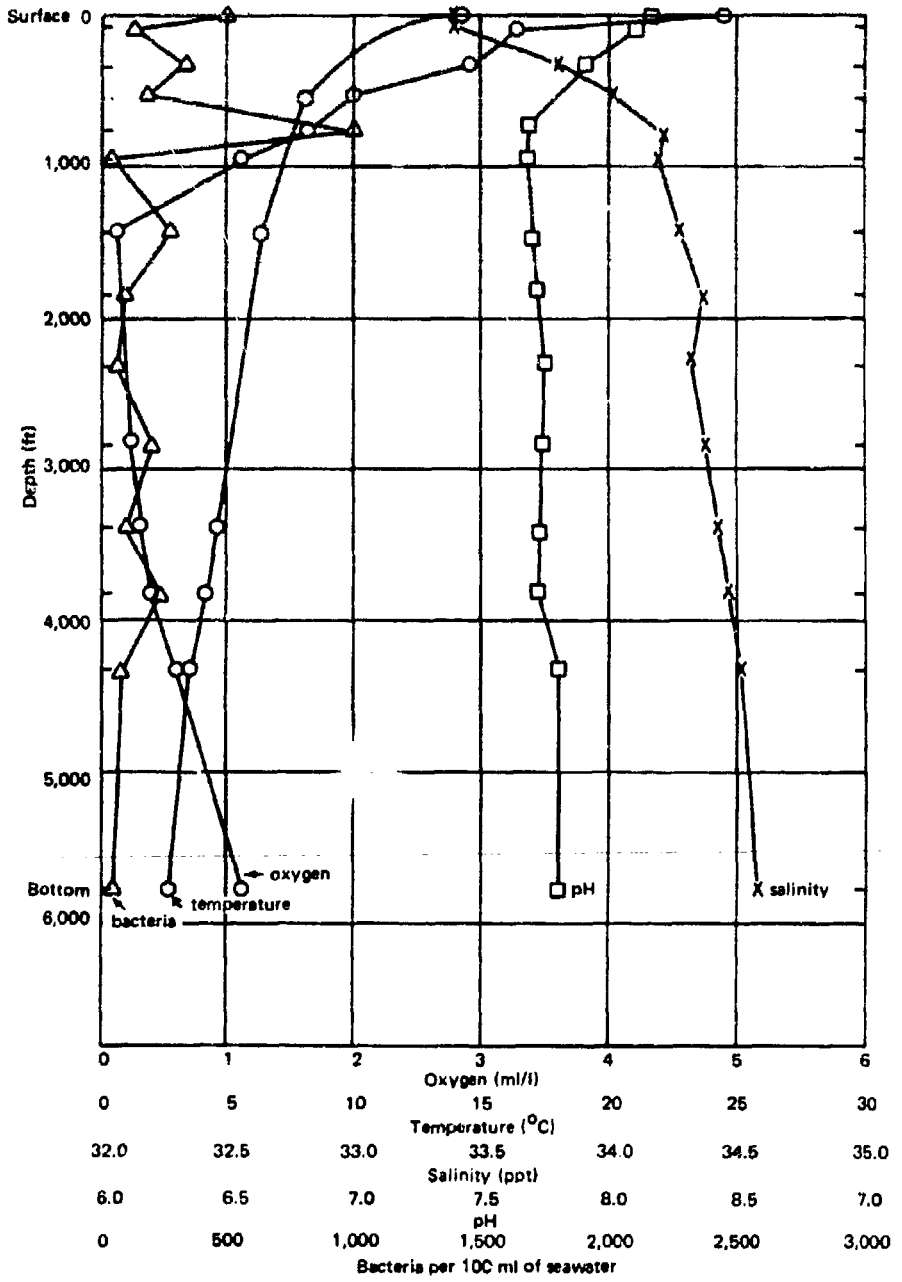

Figure 7. Relationship between vertical distribution of bacteria and environmental parameters (Station 7, Cruise A612-1, December 1966).

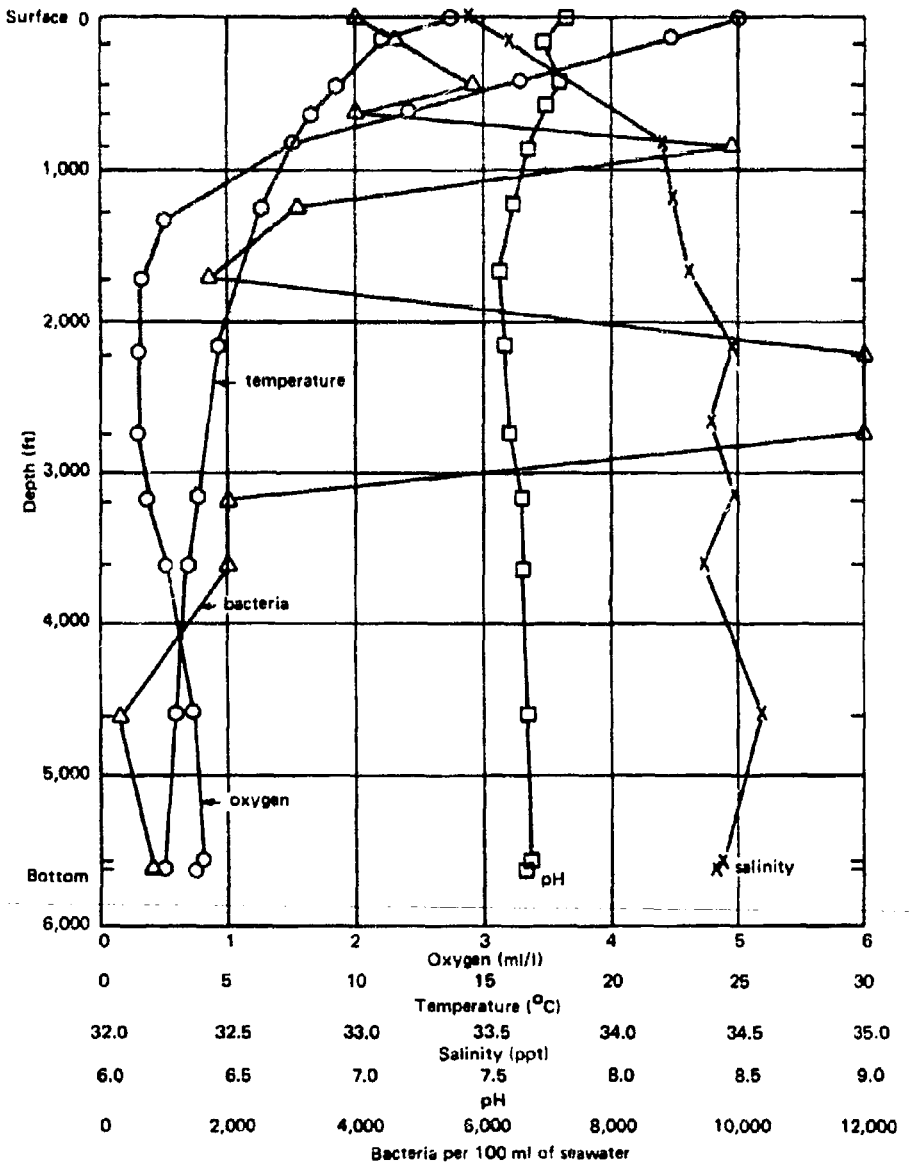

Figure 8. Relationship between vertical distribution of bacteria and environmental parameters (Station 7, Cruise A706-1, June 1967).

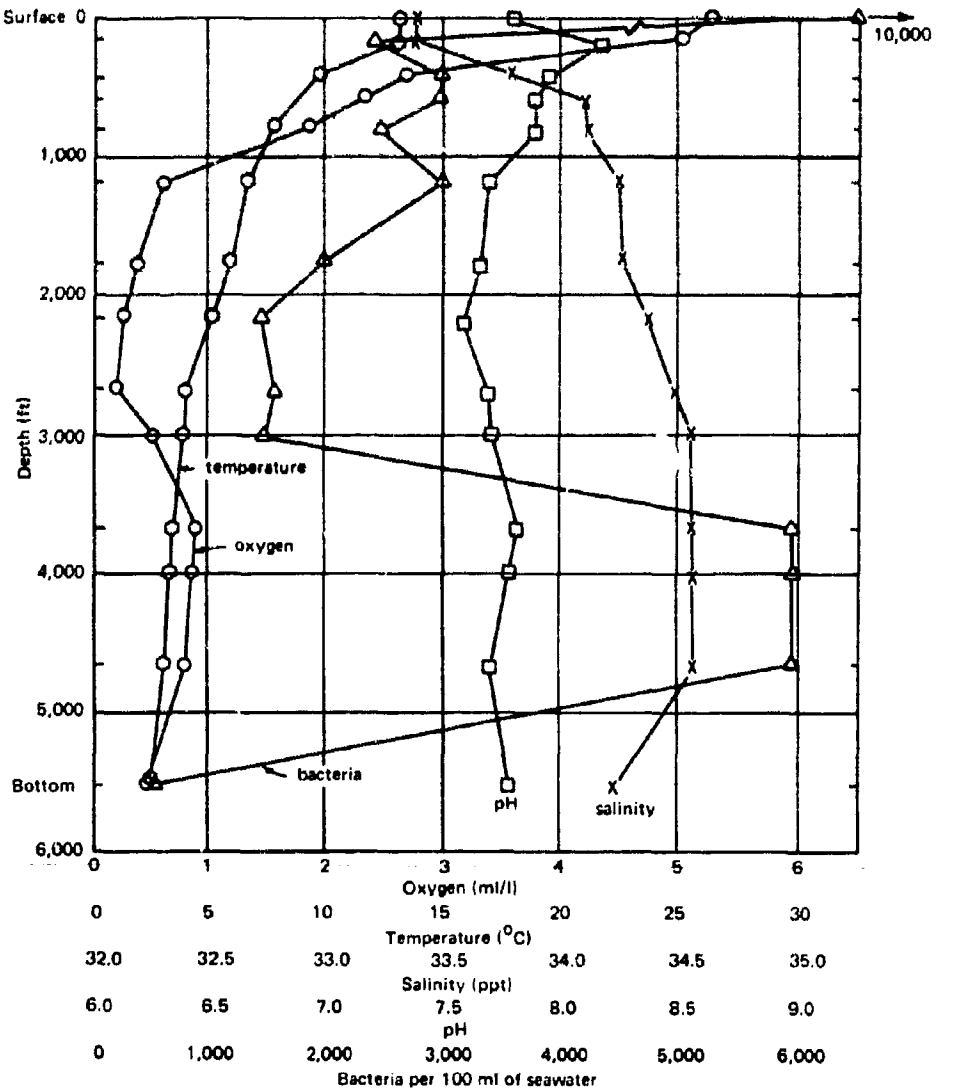

Figure 9. Relationship between vertical distribution of bacteria and environmental parameters (Station 7, Cruise A706-1, June 1967).

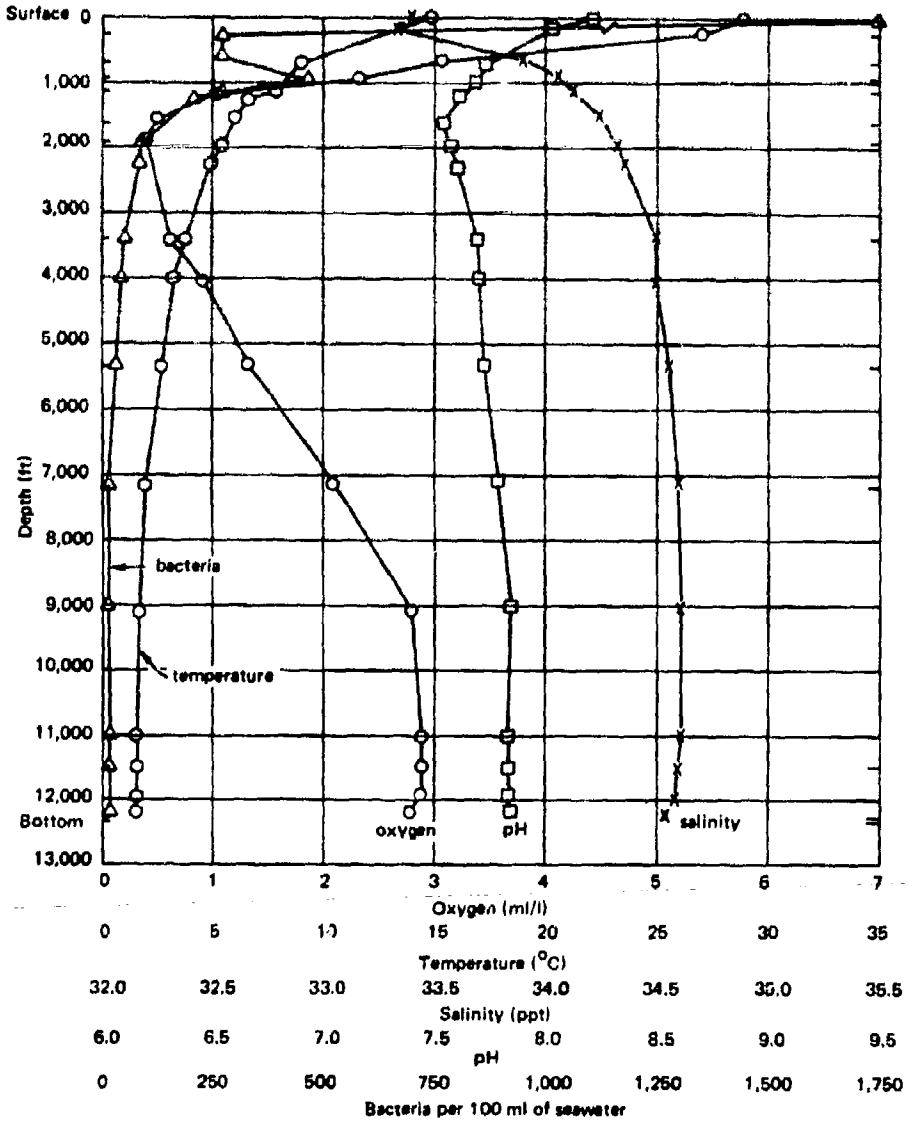

Figure 10. Relationship between vertical distribution of bacteria and environmental parameters (Station 10, Cruise A601-1, January 1966).

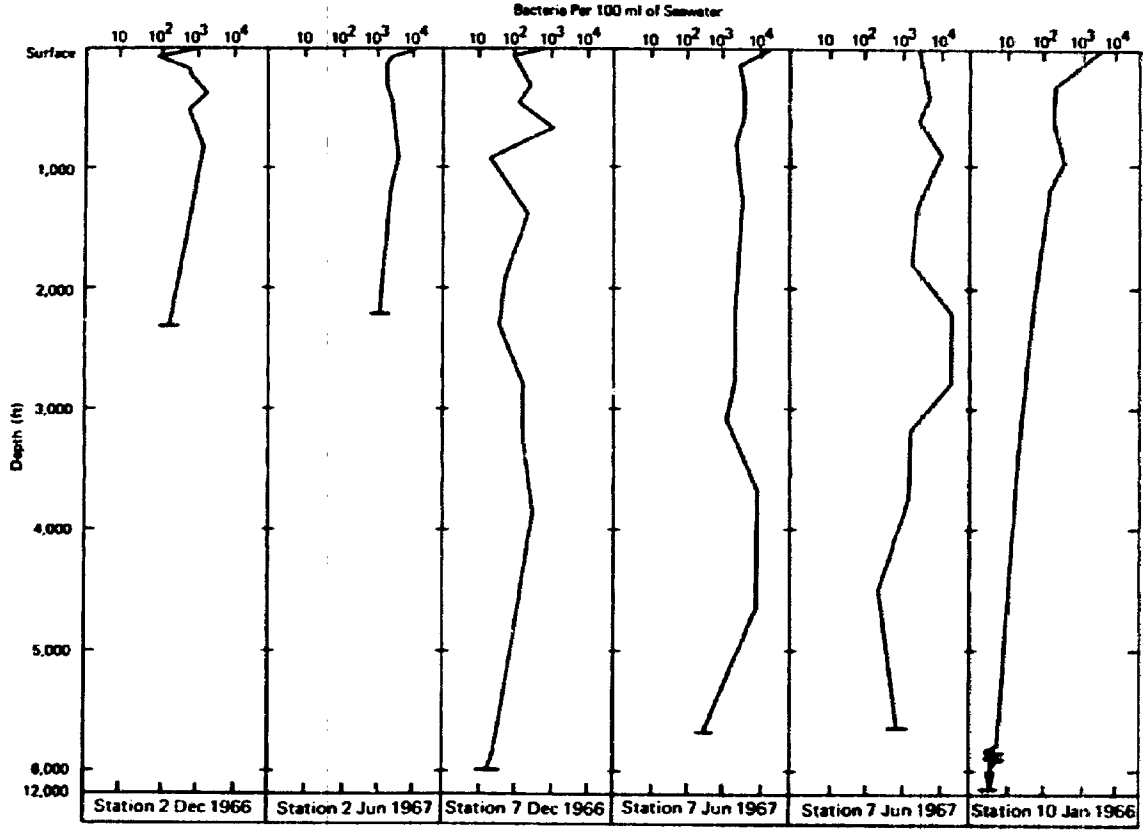

Figure 11. Vertical distribution of bacteria at NCEL oceanographic stations off the Santa Barbara Channel Islands.

Figure 12. Gram-positive microbes found at NCEL oceanographic stations off the Santa Barbara Channel Islands at a depth of 2,500 feet (magnified).

Five separate bacterial colonies which appeared to be quite different from one another in shape and pigmentation were also selected for staining and physiological tests. These bacteria were collected with sterile bacteriological samplers in June 1967 at station 7 between the depths of 1,500 and 5,600 feet. All of the bacteria were gram-negative (red). Some bacterial cells were of very irregular shape and form as shown in Figures 13 and 14. Four of the five bacterial species were not able to grow in a nutrient broth made with distilled water, but grew very well in a nutrient broth made with seawater. Only one species of this group was able to grow in nutrient broths made with distilled water and seawater, respectively. The deep ocean bacterial species collected in these samples were not identified.

A sharp increase in the bacterial count occurred in a water sample collected at the sea floor with a bottom-actuated sampling device (Figure 6). This increase was attributed to the presence of small amounts of bottom sediment collected in the Fjarlie bottle when it was tripped on the sea floor. As a result, the majority of the bacteria isolated from this bottle on the membrane filter were benthic microorganisms normally found inhabiting the sediment.

Figure 13. Gram-negative microbes found at NCEL oceanographic stations off the Santa Barbara Channel Islands at a depth of 1,250 feet (magnified).

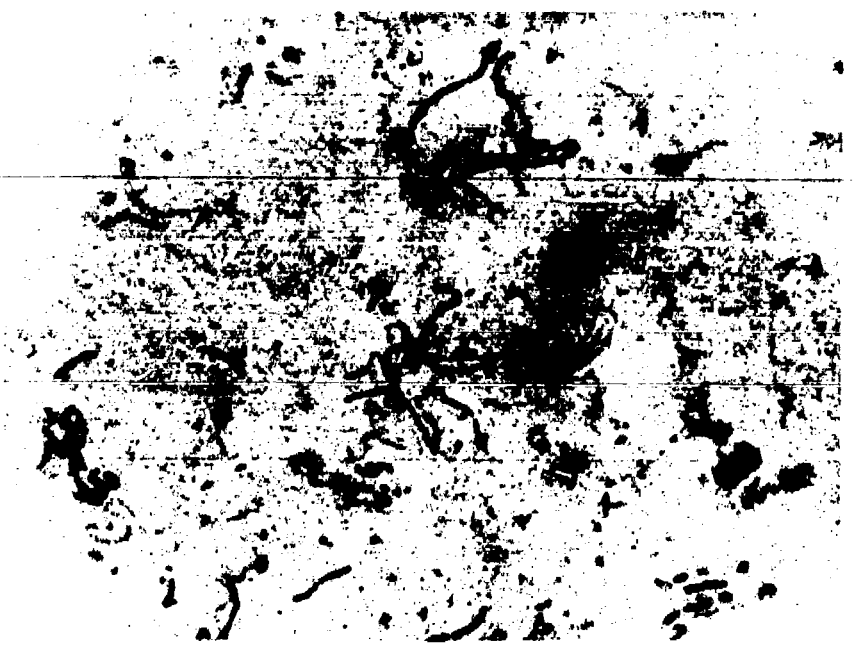

Figure 14. Gram-negative, filamentous microbes found at NCEL oceanographic stations off the Santa Barbara Channel Islands at a depth of 1,250 feet (magnified).

The engineering properties of marine sediments taken from this area (oceanographic station 2) are reported in Reference 6. The results of carbonate-organic carbon analysis showed that the average carbonate content was 12.78% (range of 1.53 to 23.36%) and the average organic content was 1.35% (range of 0.86 to 2.14%).

Sediment Samples

The sediment samples reported herein were obtained in June 1967 during Cruise A706-1 aboard the ***USNS DAVIS (AGOR-5)***. Bacteriological analyses of sediment and core samples obtained previously from various STU sties have been conducted and the results have been reported in Reference 7.

Table 1 shows the number of aerobic and anaerobic bacteria at various depths from the surface of core samples taken at oceanographic station 2. The number of aerobic bacteria found in the surface layer of the core sample ranged from 20,000 to 55,000 per gram of wet sediment. The number of aerobic bacteria found 3 inches below the surface ranged from 200 to 1,000 per gram of wet sediment, and the number dropped to 200 to 400 per gram of wet sediment at a depth of 6 inches below the surface of the core sample. The number of anaerobic bacteria was low compared to the number of aerobic bacteria present in the upper section of the core. However, about 12 inches below the surface of the sediment, the anaerobic bacterial count was higher than the aerobic bacterial count. Sulfate-reducing bacteria were present throughout the core samples. The largest numbers of sulfate-reducers were found at the 6-inch and 18-inch strata of one core sample. This was indicated by the rapid production of black sulfides in the sealed test tubes containing M10E medium as compared to other test tubes containing samples from other strata. The presence of sulfate-reducing bacteria in the sediment samples was of particular interest because these bacteria are considered to be responsible for the anaerobic corrosion of metals.[8,9]

In other bacteriological analyses of long cores of marine sediment, marine bacteria have been found about 100 inches below the surface in core samples obtained from depths of about 3,000 feet of water.[10]

It was demonstrated that the highest numbers of viable bacteria were found in samples obtained with a modified ZoBell bacteriological sampler at the sediment-seawater interface as a mixture of sediment and seawater. The highest number of aerobic bacteria determined by pour-plating and counting bacteria on nutrient agar plates (medium 2216E) was over 2,000,000 per gram of wet sediment.[11]

Table 1. Bacteriological Analysis of Core and Sediment Samples From Station 2[1]

Location of Station: 34°06'N 120°42'W Cruise A706-1 June 1967

Core[2]	Depth (in.)	Bacteria in 1 Gram of Wet Sediment	
		Aerobic	Anaerobic
C-1	Surface	55,000	6,000
	3	200	300
	6	200	20
	13	0	650
C-2	Surface	20,000	7,000
	3	1,000	500
	6	400	20
	12	0	10
	18	10	450
Bottom Sediment[3]		160,000	70,000

1/ Sulfate-reducing bacteria were present in all sediment samples.
2/ Sample cores were 1.5 inches in diameter.
3/ Sample taken with a steel pipe dredge.

In sediment samples obtained with a pipe dredge over oceanographic station 2 in 2,400 feet of water, the highest aerobic bacterial count obtained was 160,000 per gram of wet sediment. The highest anaerobic bacterial count obtained was about 75,000 per gram of wet sediment.

Sterile Versus Nonsterile Samplers

Information about the number of bacteria obtained with sterile bacteriological water samplers versus nonsterile Nansen and Fjarlie bottles was inconclusive. Generally, the bacterial count was higher for nonsterile samplers than for sterile bacteriological samplers. However, there were water samples in which identical numbers of bacteria were isolated from

each type of sampler, and sometimes water from the nonsterile samplers contained fewer bacteria than water from the sterile samplers (Table 2). Kriss[12] has found that water samples collected simultaneously at the same depth in the Black Sea in unsterilized Nansen and sterilized microbiological bottles contained practically the same number of bacteria.

Table 2. Oceanic Bacteria Collected With Sterile and Unsterile Water Samplers at NCEL Stations

(Samples were not all collected on the same day)

Depth (ft)	Type of Sampler Used		Number of Bacteria Per 100 ml of Seawater
	Sterile	Unsterile	
845		x	700
855	x		1,230
1,230	x		1,800
1,240		x	1,200
2,221		x	7
2,226	x		3
2,335		x	400
2,343	x		160
2,874	x		1
2,879		x	24
3,195		x	15
3,200	x		0
3,592		x	1
3,600	x		0
4,100		x	40
4,105	x		10
4,590		x	1,500
4,600	x		1,500
6,726		x	28
6,736	x		10
12,017		x	2
12,027	x		0
12,210		x	35
12,220	x		22

Effect of Pressure on Bacteriological Samplers

When water samples were taken at 6,800 and 12,000 feet with the bacteriological samplers, a small puncture was detected in some of the pear-shaped rubber bulbs and also in some of the 3/16-ID x 1/8-wall thickness x 9-inch-long latex rubber surgical tubings upon recovery. The high hydrostatic pressure encountered at great depth forced the side of the rubber bulb into the open end of the 1-1/2-inch-long x 5/32-inch-ID glass tube which was used to connect the rubber bulb and the surgical tubing. One end of the surgical tubing where a glass tube (sealed at one end) was inserted also ruptured at great depth on several occasions. Such phenomena caused premature collection of the water samples from unknown depths. ZoBell[13] reports similar failure in rubber tubing during the Danish Galathea Deep-Sea Expedition.

The rubber tube failures were corrected by filling the glass tube (sealed at one end) with sterile seawater or distilled water before use, and the rubber bulb failures were corrected by inserting a 1/8-ID x 3/32-wall-thickness x 9-inch-long natural rubber tubing (not latex surgical rubber tubing) directly into the opening of the rubber bulb and then sealing the connection with a rubber cement.

Deep Ocean Algae

When a test rack (STU II-2) was recovered from depth of 2,370 feet,[14, 15] the surfaces of some of the metal specimens such as aluminum, K-Monel, and a Monel 400 bolt had numerous pits filled with corrosion products. Chemical analysis conducted on the corrosion products formed in these pits revealed the presence of a low to moderate amount of algae and some diatoms in addition to metal oxides.

Finding algae at this depth in the Pacific Ocean was surprising. The microscopic plants found in the corrosion products could be a species of blue-green algae (Cyanophyceae); these algae are reported to be found in large numbers between 1,300 and 13,000 feet in the Atlantic and Mediterranean.[16]

DISCUSSION

Adverse environmental conditions at great depths in the ocean, such as near-freezing water temperature, high hydrostatic pressure, and total darkness, might be expected to slow bacterial activity. However, the deep-sea microorganisms were found to be very active even at a depth of 5,000

feet when given suitable substrate for multiplication.[7, 11, 14] For example, when natural fibers in the form of cotton and Manila rope, and burlap (some covered with coal tar) were exposed on the sea floor, the materials were quickly destroyed by the activity of marine microorganisms. Heavy slime growth composed of microorganisms was also found covering the entire surface of a flexible vinyl tube. The plastic tube contained chemical compounds (plasticizers) which served as a substrate for the growth of these microorganisms. Examination of the vinyl tube showed that it had lost its flexibility and had shrunk.[7]

Any engineering materials, such as metals, plastics, elastomers, paints, and protective coatings placed in the sediments and in seawater where marine microorganisms are present will be adversely affected. Since marine bacteria are the first marine organisms to attach to submerged objects, they play an important role in the fouling of submerged surfaces by (1) affording a foothold for other animals, (2) discoloring glazed or bright surfaces, (3) serving as a source of food for barnacles, etc., and (4) promoting the early deposition of the calcareous cements of sessile animals.[5] It is not possible to state which species of bacteria are of significance to the structural designer; however, it is known that sulfate-reducing anaerobic bacteria are of significance. The degree of significance, particularly with respect to hydrogen embrittlement of high-strength steels which are under stress, depends upon the population of anaerobic bacteria and the amount of H_2S (hydrogen sulfide) generated. Cases of failure of deep oil well casings by the H_2S produced by these bacteria are well known.

CONCLUSIONS

The variation in the bacterial population with depth does not seem to be influenced by any single environmental factor. However, the combined effects of water temperature, undersea currents, and availability of food could be responsible for variations in the vertical distribution of bacteria in the ocean environment.

REFERENCES

1. A. E. Kriss. Marine microbiology, deep sea. New York, Interscience (Wiley), 1963.

2. R. Y. Morita and C. E. ZoBell. "Occurrence of bacteria in pelagic sediments collected during the Mid-Pacific Expedition," Deep-Sea Research, vol. 3, no. 1, Oct. 1955, pp. 65-73.

3. U. S. Navy Hydrographic Office. H. O. Publication no. 607: Instruction manual for oceanographic observations, 2nd ed. Washington, D. C., 1955.

4. U. S. Naval Civil Engineering Laboratory. Oceanographic data reports, Dec. 1965 to Feb. 1966. Port Hueneme, Calif.

5. C. E. ZoBell. Marine microbiology; a monograph on hydrobacteriology. Waltham, Mass., Chronica Botanica, Company, 1946.

6. U. S. Naval Civil Engineering Laboratory. Technical Report R-503: Engineering properties of marine sediments near San Miguel Island, California, by M. C. Hironaka. Port Hueneme, Calif., Dec. 1966. (AD 644192)

7. ———. Technical Report R-428: Deep-ocean biodeterioration of materials -- Part III. Three years at 5,300 feet, by J. S. Muraoka. Port Hueneme, Calif., Feb. 1966. (AD 631078)

8. R. L. Starkey. "The general physiology of the sulfate-reducing bacteria in relation to corrosion," Producers Monthly, vol. 22, no. 8, June 1958, pp. 12-30.

9. A. W. Baumgartner. "Sulfate-reducing bacteria -- their role in corrosion and well plugging," Producers Monthly, vol. 26, no. 7, July 1962, pp. 2-5.

10. S. C. Rittenberg. "Bacteriological analysis of some long cores of marine sediments," Journal of Marine Research, vol. 3, no. 3, 1940, pp. 191-201.

11. U. S. Naval Civil Engineering Laboratory. Technical Report R-329: Deep-ocean biodeterioration of materials -- Part I. Four months at 5,640 feet, by J. S. Muraoka. Port Hueneme, Calif., Nov. 1964. (AD 608939)

12. A. E. Kriss, M. N. Lebedeva, and A. V. Tsiban. "Comparative estimate of a Nansen and microbiological water bottle for sterile collection of water samples from depths of seas and oceans," Deep-Sea Research, vol. 13, no. 2, Apr. 1966, pp. 205-212.

13. C. E. ZoBell. "Some effects of high hydrostatic pressure on apparatus observed on the Danish Galathea Deep-Sea Expedition," Deep-Sea Research, vol. 2, no. 1, 1954, pp. 24-32.

14. U. S. Naval Civil Engineering Laboratory. Technical Report R-525: Deep-ocean biodeterioration of materials – Part VI. One year at 2,370 feet, by J. S. Muraoka. Port Hueneme, Calif., May 1967. (AD 651124)

15. ———. Technical Report R-504: Corrosion of materials in hydrospace, by F. M. Reinhart. Port Hueneme, Calif., Dec. 1966. (AD 644473)

16. N. B. Marshall. Aspects of deep sea biology. London, Hutchinson & Company, Ltd., 1958.

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13 ABSTRACT

The vertical distribution of bacteria and its relationship to the environmental parameters from the surface of the sea to a depth of 12,000 feet was studied off the coast of Southern California near the Santa Barbara Channel Islands. The bacterial population varied randomly with location and depth. The highest bacterial count (12,000 per 100 ml of seawater) was found at depths between 2,300 and 2,700 feet in the minimum oxygen zone. The lowest number of bacteria (about 5 per 100 ml of seawater) was found near the sea floor at a depth of 12,000 feet. The variation of bacterial population density with depth does not seem to be influenced by any single environmental factor. This report also presents data on the bacteriological analysis of sediment samples, a comparison of the number of bacteria present in seawater samples collected with sterile bacteriological samplers and nonsterile water samplers, and incidence of failures of rubber components of bacteriological samplers.

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	ROLE	WT	ROLE	WT	ROLE	WT
Bacteria						
Aerobic						
Anaerobic						
Sulfate reducing						
Bacteriology						
Water samples						
Sediment samples						
Nansen bottle						
Fjarlie bottle						
ZoBell sampler						
Cobet sampler						
Metal deterioration						
Plastics deterioration						
Rubber failure						